## REMARKS

Claims 1- 19 and 21-53 are pending in the application. Claims 33-51 and 53 are withdrawn from consideration. Claims 1 and 17 have been amended to better clarify what Applicants regard as the invention. Support for the amendment can be found throughout the specification, but particularly in Example 2 on pages 17-19. No new matter has been added by way of this amendment. Thus, as a result of the foregoing amendment, claims 1-19 and 21-53 remain pending. Reconsideration of this application is respectfully requested.

Applicants' representatives would like to express their sincere appreciation for the telephonic discussion held with Examiner Wehbe on January 25, 2005 as related to the claims under consideration. Examiner Wehbe recommended that Applicants should consider filing a Request for Continued Examination in order to have the current claims as amended entered into the application. Furthermore, Examiner Wehbe also recommended submitting a Declaration under 37 C.F.R. 1.132. Accordingly, a Request for Continued Examination and a Declaration under 37 C.F.R. 1.132 are submitted concurrently herewith, and entry of the current amendment is believed to be in order and is requested.

### *Rejection under 35 U.S.C. §112, second paragraph*

Claims 1-19, and 21-32 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite. In particular, the Examiner alleges that the polynucleotide sequence which is expressed is the same polynucleotide sequence contained in the construct. However, as written, the claims appear to be referring to two different polynucleotide sequences, one which encodes a heavy chain IgG1 immunoglobulin gene and a detectable protein, and a second which encodes and expresses any immunoglobulin light chain or heavy chain fused to a detectable protein. The Examiner asserts that if the polynucleotide sequence in the construct and the polynucleotide sequence expressed are intended to be one and the same, then the limitations attributed to the expressed polynucleotide are in conflict with those of the polynucleotide in the genetic construct since the construct polynucleotide comprises immunoglobulin heavy chain sequences,

particularly the CH1, CH2 and G1 heavy chain constant region exons, not kappa light chain or lambda light chain sequences, or other heavy chain gene sequences such as the IgM, IgD or IgA constant region exons.

The Examiner further alleges that claims 2-5 are confusing in that they refer to "said fusion polynucleotide" in claim 1. However, claim 1 recites two polynucleotides, neither of which are identified as a "fusion polynucleotdide". Thus, it is unclear to which polynucleotide these claims refer.

Applicants respectfully traverse the Examiner's rejection and herein amend claim 1 to better clarify the relationship between the genetic construct and the expressed protein. Furthermore, the claim has also been amended to recite "...a genetic construct comprising a fusion polynucleotide..." Accordingly, Applicants respectfully request withdrawal of the rejection.

***Rejection under 35 U.S.C. §102(b)***

Claims 17-19 and 22-25 have been rejected under 35 U.S.C. 102(b) as being anticipated by Fell et al. in U.S. patent No. 5,202,238. Applicants respectfully traverse the Examiner's rejection for the following reasons.

Fell et al. teach how an immortalized *in vitro* cell line that has been generated by a fusion of a myeloma and spleen cell that produces antibodies can be genetically modified with two polynucleotide plasmids so that a component of an immunoglobulin gene has been replaced with a portion of the human variable or constant gene linked to an enzyme or substrate such as beta galactosidase, alkaline phosphatase or horseradish peroxidase. Fell et al teach that the antibodies produced by these cells can be used as labeled antibodies in diagnostic assays without further modification. Furthermore, Fell et al teach that the replacement gene can be inserted into either or both the light or heavy chain immunoglobulin genes. Furthermore, Fell et al. provide an embodiment whereby the replacement gene encodes all or a portion of IgG1, thus having a linked enzyme present in exon G1. The Examiner alleges that Fell et al teach cells with the same structural and functional limitations as the cells recited in claims 17-19 and 22-25.

Applicants assert that the cells taught by Fell, et al. are immortalized e.g.

tumorigenic cells that produce just a single antibody. Furthermore, the Fell et al. reference teaches that the method for producing such cells is accomplished using a two vector system. The use of such a system is flawed in that each individual gene is under the control of different promoters and the resulting product may not contain the desired characteristics, e.g. specific antigen binding or affinity, correct assembly or secretion.

As noted previously, Applicants have identified a means for modifying the stem cells of a non-human animal such that all B cells, after their normal somatic recombination, carry a genomic sequence that has a fusion polynucleotide appended to a very specific location in the light chains or heavy chains such that this location is demonstrated to have no effect on the synthesis, assembly, transport of the antibodies or generation of an antibody response. Thus, this system can be used for the generation of a detectable antibody specific for a preselected antigen ***in vivo,*** and have thus attained a diversity of antibodies, a more accurate means of achieving the desired immune response, and generating antibodies where the fusion antibody retains full binding properties. The more random recombination in preselected in vitro cell lines cannot generate a diversity and due to the random nature of recombination, the locus cannot be prescribed and therefore the nature of the fusion is variable and unpredictable.

Moreover, as noted in the present application, embryonic stem cells are first transfected with a ***single*** targeting vector containing the immunoglobin region and the detectable protein region construct, that is, a ***fusion polynucleotide***. The expression of the gene product derived from such a construct would be better controlled since the entire genetic construct for the immunoglobulin and the detectable protein are under the control of the same and more appropriate promoters. These ES cells are then injected into blastocysts and implanted into female non-human mammals. The resulting chimeric non-human mammals are used to parent non-human mammals. Two heterozygous non-human mammals are then used to produce homozygous non-human mammals. Accordingly, upon injection of a preselected antigen, the non-human mammals will produce antibody specific for the antigen and will also contain the detectable protein. Moreover, the cells obtained from these animals are primary antibody producing cells, that is, non-tumorigenic cells, unlike the hybridomas taught by Fell et al. Furthermore, rather than

engineering the immortalized (tumorigenic) antibody producing cells ***in vitro*** as demonstrated by Fell et al., Applicants have engineered the non-human mammals to produce the desired antibody producing cell ***in vivo***. Furthermore, as Fell et al. note in column 11, lines 50-55, the antibodies produced using their *in vitro* approach may be used for *in vivo* targeting of enzymes, toxins, drugs etc. **Fell et al. neither teach nor suggest production of a cell containing a fusion polynucleotide using a single targeting vector.** The methods disclosed and claimed by **Fell et al. clearly utilize a two vector system.** Fell et al. neither teach nor suggest production of a cell with a prescribed fusion of a plasmid in the genome of the cell such that all antibodies produced by somatic recombination will contain the fusion construct. Furthermore, Fell et al. do not teach that the chimeric antibodies produced *in vitro* contain a flexible linker sequence between the immunoglobulin component and the detectable protein component where the flexible linker is important for maintaining the physiological efficacy of the antibody. Applicants assert that the claims of the instant application, as currently amended, are not anticipated by Fell et al. Withdrawal of the rejection is respectfully requested.

***Rejection under 35 U.S.C. §103***

**A. Fell et al. in view of Casey et al.**

The Examiner's rejection of claims 20-21 and 27-30 under 35 U.S.C. §103 as being unpatentable over Fell et al. (U.S. patent No. 5,202,238) in view of Casey et al. (June 2000, Prot Engineer Vol. 13(6): 445-452) is maintained.

The Examiner has the initial burden of establishing a *prima facie* case of obviousness. A finding of obviousness under § 103 requires a determination of the scope and content of the prior art, the differences between the claimed invention and the prior art, the level of ordinary skill in the art, and whether the differences are such that the claimed subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. Graham v. Deere, 383 US 1 (1966). Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion that the combination be made. In re Stencel, 828 F2d 751, 4 USPQ2d 1071 (Fed. Cir. 1987). Furthermore, there must be a

reasonable expectation of success for a rejection under § 103 to be proper.

**The invention as claimed.** As noted previously, claim 20 has been canceled, and the dependency of claim 21 has been amended to now depend from currently amended claim 17. Furthermore, claim 17 has been amended to recite a cell having a single vector. Claim 27 was previously amended to read on the genetically-modified immune cell obtained from the genetically modified non-human mammal, which contains at least one detectable protein or peptide which is an autofluorescent protein or peptide, a visibly-detectable protein or peptide, an enzymatically active protein or peptide, a protein or peptide capable of interacting with another molecule to produce a detectable product, wherein said protein or peptide capable of interacting with another molecule to produce a detectable product is selected from the group consisting of an intein, a biotin-binding subunit of streptavidin or avidin, a His tag, a chitin-binding domain, or any combination thereof. The claims depending from claim 27 were previously amended to recite various detectable proteins which are encoded by the genes comprising the chimeric antibody.

**The Fell et al. reference as a whole.** As noted above, Fell et al teach genetically modified antibody producing cells which have undergone homologous recombination ***in vitro*** to replace a component of the immunoglobulin genes with all or a portion of a human variable or constant gene linked to an enzyme or substrate such as beta galactosidase, alkaline phosphatase or horseradish peroxidase. Fell et al teach that the antibodies produced by these cells can be used as labeled antibodies in diagnostic assays without further modification. Furthermore, Fell et al teach that the replacement gene can be inserted into either or both the light or heavy chain immunoglobulin genes. Furthermore, Fell et al. provide an embodiment whereby the replacement gene encodes all or a portion of IgG1, thus having a linked enzyme present in exon G1.

As noted above, Applicants assert that the cells taught by Fell, et al. are immortalized eg. tumorigenic antibody producing cells. Furthermore, the Fell et al. reference teaches that the method for producing such cells is accomplished using a two vector system. The use of such a system is flawed in that each individual gene is under

the control of different promoters and the resulting product may not contain the desired characteristics, eg. specific antigen binding or affinity, correct assembly or secretion. Fell et al. do not teach or suggest use of a ***single*** targeting vector containing the immunoglobin region and the detectable protein region construct, that is, a ***fusion polynucleotide***. Fell et al. do not teach or suggest use of a bacterial artificial chromosome for preparation of and insertion of their construct into the immune cell.

Applicants assert that **Fell et al. neither teach nor suggest preparation of a cell containing a fusion polynucleotide encoding an immunoglobulin gene and a detectable protein using a single targeting vector.** Furthermore, Fell et al. do not teach or suggest that the chimeric antibodies produced *in vitro* should contain a flexible linker sequence between the immunoglobulin component and the detectable protein component. Fell et al. do not teach nor suggest the preparation or production of genetically modified antibody producing cells ***in vivo***, nor do Fell et al teach or suggest the possible variations of the detectable proteins envisioned by the present application, as claimed in previously presented claim number 27.

**Casey et al. reference as a whole.** Casey et al. describe the construction of a single chain antibody *in vitro* in a bacterial system. This antibody contains a flexible glycine linker and GFP.

As noted previously, Casey et al. do not teach or suggest the production of antibody producing cells *in vivo*, nor do Casey et al. teach or suggest the particular detectable proteins now claimed in previously presented claim number 27. Furthermore, as one skilled in the art can appreciate, the procedures for preparing an antibody molecule in a bacterial system do not allow for correct post-translational modification of the antibody molecule, such as glycosylation, which is needed for maximizing function and specificity. Accordingly, Applicants assert that such differences between the prokaryotic and eukaryotic systems would not motivate one skilled in the art to combine the teachings of Casey et al. with those of Fell et al.

Furthermore, Casey et al. do not teach or suggest preparation of fusion polynucleotides in immune cells containing a large immunoglobulin gene and a detectable protein. In fact, the teachings by Casey et al. teach away from the present invention for the following reasons. One of skill in the art, upon review of the Casey reference, would not be motivated to proceed with the preparation of the fusion polynucleotide of the present invention due to the size of the fusion construct. In fact, Casey et al. use a **single chain antibody variable fragment** (scFv) for combining with the detectable label. The teachings by Casey et al. do not motivate nor suggest that the larger molecules prepared by the methods of the present invention could be effective. There is simply no motivation or reasonable expectation of success in making such molecules. In fact, Casey et al. discuss the potential problems with folding of the molecule in the proper configuration to generate a fully functional fluorescent scFv. This would lead one skilled in the art to believe that such problems would in fact be encountered even more so by using the full immunoglobulin gene fused to a gene encoding a detectable marker protein, as taught by the present invention. As such, Casey et al. teach away from the present invention. Applicants assert that given these facts, one skilled in the art would not be motivated to combine the teachings of Casey et al. with that of Fell et al. to produce the fusion polynucleotides of the present invention.

**The analysis under § 103(a).** When the teachings of Fell et al. are combined with the teachings of Casey et al., Applicants assert that one of skill in the art would not be motivated to produce the fusion polynucleotides of the present invention as presently claimed for the following reasons. As noted above, Casey et al. utilize a prokaryotic system to generate a detectably labeled **single chain antibody fragment**. Casey et al. specifically state the difficulties of such an endeavor due to the possible loss of functionality in the detectably labeled single chain antibody fragment. Upon reading the authors statement regarding these potential problems, Applicants assert that one of skill in the art would not be motivated to attempt the preparation of an immune cell comprising a fusion polynucleotide which might contain a larger immunoglobulin (not a fragment as taught by Casey et al.) fused to a detectable protein because there would not

be a reasonable expectation of success. Thus, while Fell et al. teach how to prepare an immortalized antibody producing cell using various vectors to target the genes of interest, there would be no motivation to do so given the alleged potential problems in folding of the proteins while at the same time maintaining functionality of the protein components.

Accordingly, Applicants assert that there would be no motivation to combine the teachings of Casey et al. with that of Fell et al., which would then result in the particular aspects of the present invention, in particular, the cells, as currently claimed.

In light of the foregoing arguments and claim amendments, Applicants respectfully request withdrawal of this rejection.

**B. Fell et al. in view of Casey et al. and further in view of Rajewsky et al.**

Furthermore, the rejection of claims 1-9, 11-14 and 52 under 35 U.S.C. §103 as being unpatentable over Fell et al. , U.S. patent No. 5,202,238, in view of Casey et al. (June 2000) Prot Engineer. Vol. 13(6): 445-452 and further in view of Rajewsky et al., U.S. Patent No. 6,570,061, is maintained.

**The invention as claimed.** Claim 1 as amended claims a genetically-modified non-human mammal containing a genetic construct having a single vector comprising a fusion polynucleotide, said fusion polynucleotide comprising an immunoglobulin component selected from the group consisting of the kappa immunoglobulin light chain, the lambda immunoglobulin light chain, an immunoglobulin heavy chain, and any combination thereof, and at least one detectable protein, wherein said fusion polynucleotide sequence is inserted into a cell of said non-human mammal using a single targeting vector. The non-human mammal is capable of expressing at least one chimeric immunoglobulin gene comprising a polynucleotide sequence encoding at least one detectable protein or peptide fused with a gene expressing an immunoglobulin component selected from the group consisting of the kappa immunoglobulin light chain, the lambda immunoglobulin light chain, an immunoglobulin heavy chain, and any combination thereof, wherein antibodies secreted by immune cells of said genetically-modified mammal comprise said at least one detectable protein or peptide. The dependent claims point out the particular aspects of

where the gene encoding the detectable protein may be located in the fusion construct, as well as the various types of detectable proteins or combinations thereof which are contemplated for inclusion in the fusion polynucleotide.

**The Fell et al. reference as a whole.** As noted above, Fell et al teach genetically modified antibody producing cells (having two vectors) which have undergone homologous recombination ***in vitro*** to replace a component of the immunoglobulin genes with all or a portion of a human variable or constant gene linked to an enzyme or substrate such as beta galactosidase, alkaline phosphatase or horseradish peroxidase. Fell et al teach that the antibodies produced by these cells can be used as labeled antibodies in diagnostic assays without further modification. Furthermore, Fell et al teach that the replacement gene can be inserted into either or both the light or heavy chain immunoglobulin genes. Furthermore, Fell et al. provide an embodiment whereby the replacement gene encodes all or a portion of IgG1, thus having a linked enzyme present in exon G1.

Fell et al. do not teach or suggest the preparation of a fusion polynucleotide comprising an immunoglobulin gene and a detectable protein for insertion into non-human mammals using the methods described in the present application. Furthermore, Fell et al. do not teach or suggest use of a ***single*** targeting vector containing the immunoglobin region and the detectable protein region construct, that is, a ***fusion polynucleotide*** for insertion into a cell of a non-human mammal. Fell et al. do not teach or suggest use of a bacterial artificial chromosome for preparation of and insertion of their construct into the immune cell of said non-human mammal. Furthermore, Fell et al. do not teach or suggest that the chimeric antibodies produced by said non-human mammal should contain a flexible linker sequence between the immunoglobulin component and the detectable protein component. Fell et al. do not teach nor suggest the preparation or production of genetically modified antibody producing cells ***in vivo***, nor do Fell et al teach or suggest the possible variations of the detectable proteins envisioned by the present application.

**Casey et al. reference as a whole.** Casey et al. describe the construction of a single chain antibody *in vitro* in a bacterial system. This antibody contains a flexible glycine linker and GFP.

Casey et al. do not teach or suggest the preparation of a fusion polynucleotide comprising an immunoglobulin gene and a detectable protein for insertion into non-human mammals using the methods described in the present application. Furthermore, Casey et al. do not teach or suggest use of a ***single*** targeting vector containing the immunoglobin region and the detectable protein region construct, that is, a ***fusion polynucleotide*** for insertion into a cell of said non-human mammal. Furthermore, as one skilled in the art can appreciate, the procedures for preparing an antibody molecule in the bacterial system described by Casey et al. does not allow for correct post-translational modification of the antibody molecule, such as glycosylation, which is needed for maximizing function and specificity. Such glycosylation patterns would on the other hand be accomplished using the genetically engineered non-human mammals of the present invention.

Furthermore, Casey et al. do not teach nor suggest preparation of fusion polynucleotides in non-human mammals containing a large immunoglobulin gene and a detectable protein. In fact, the teachings by Casey et al. teach away from the present invention for the following reasons. One of skill in the art, upon review of the Casey reference, would not be motivated to proceed with the preparation of the fusion polynucleotide of the present invention due to the size of the fusion construct for insertion into a cell of a non-human mammalian, such as the blastocysts of the non-human mammals of the present invention. In fact, Casey et al. use a **single chain antibody variable fragment** (scFv) for combining with the detectable label. The teachings by Casey et al. would not motivate nor suggest to one skilled in the art that the larger molecules prepared by the methods of the present invention could be effective. In fact, Casey et al. discuss the potential problems with folding of the molecule in the proper configuration to generate a fully functional fluorescent scFv. This would lead one skilled in the art to believe that such problems would in fact be encountered even more so by using the full immunoglobulin gene fused to a gene encoding a detectable marker protein,

as taught by the present invention, before insertion of such fusion polynucleotide into the non-human mammal.

**Rajewsky et al. (U.S. patent No. 6,570,061) reference as a whole.** Rajewsky et al. teach the use of homologous recombination to replace the constant region genes of the murine immunoglobulin heavy or light chain with human genes in murine embryonic stem cells and the use of these cells to make transgenic mice which produce the chimeric antibody.

Rajewsky et al do not teach the introduction of the detectable marker proteins into the chimeric immunoglobulin molecule as disclosed in the instant application, and as currently claimed. Nor do Rajewsky et al. teach an *in vivo* method of generating detectable labeled antibodies specific for a preselected antigen. In particular, Rajewsky et al. do not envision the potential for combining an enzyme label with a fluorescent label on the chimeric immunoglobulin molecule such that the antibodies so produced would serve multiple functions for research or diagnostic use.

**The analysis under § 103(a).**

1. The references even when combined do not teach the present invention.

When the teachings of Fell et al. are combined with the teachings of Casey et al., and Rajewsky et al, Applicants assert that the references even when combined do not teach or suggest the present invention. Applicants further assert that one of skill in the art would not be motivated to combine the teachings of these references to produce the genetically engineered non-human mammals of the present invention as presently claimed for the following reasons.

These references do not teach or suggest the *in vivo* production of genetically modified non-human mammals that produce the chimeric antibodies having the characteristics described and claimed in the instant application. Applicants assert that one skilled in the art would not be motivated to combine the teachings of Casey et al. with that of Fell et al. and Rajewsky et al. to produce the fusion polynucleotides of the present invention for insertion into the blastocysts of a non-human mammal. It was Applicants'

own investigative work, which identified the need for production of a genetically engineered non-human mammal which would produce detectably labeled antibodies in response to antigenic challenge *in vivo*. Furthermore, Applicants' own work recognized the benefits of creating a chimeric molecule which was engineered to have a variety of functions retained such as the ability to bind antigen, the ability to fluoresce such that the antibody can be used for diagnostic purposes, and the ability to secrete the labeled antibody without interfering with the membrane bound form of the antibody, which may inhibit targeting or processing of the protein. In addition, the Applicants of the present application recognized that one can use the genetically engineered non-human mammals repeatedly and with various antigenic challenges. It is Applicants assertion that none of the foregoing would be obvious by combining the cited references.

In addition, Applicants respectfully draw the Examiner's attention to the specification on page 11, lines 14-22, continuing on to page 12, lines 1-4; and to page 15, lines 17-21, continuing on to page 16, lines 1-11, which provide support for how the Applicants envision the insertion of the genes for the detectable protein markers into the immunoglobulin gene, thus providing a chimeric antibody molecule that contains at least one detectable protein marker. However, as noted in the specification on page 18, lines 9-17, one advantage of the present invention over the art cited is that a chimeric antibody may be prepared which may contain two different means of detection, for example, both a fluorescent marker as well as an enzyme marker, thus enabling the use of the antigen specific antibody for performing more than one function and thus may be applicable to various research and diagnostic applications, eg. the same chimeric antibody when generated with both a fluorescent label as well as an enzyme label may be used for immunohistological labeling of tissue sections, or for use for Western blot analysis or with a fluorescence activated cell sorter, or the same chimeric antibody when generated with both a fluorescent label and an intein that can be conjugated to a toxin or radiolabel can be used for tracking and therapeutic purposes. It is Applicants assertion that none of the foregoing would be obvious by combining the cited references.

2. Casey et al. teach away from the present invention.

Applicants request that the Examiner's attention be drawn to certain statements by Casey et al, for example, on page 1, the third paragraph under the Introduction, whereby the authors attest to the potential problems with proper folding of the detectably labeled single chain antibody variable fragment to retain proper functionality. Casey et al. state:

"The design of an EGFP-scFv chimera posed some interesting questions regarding protein folding."

Applicants assert that the construct of Casey et al. is expressed in a prokaryotic organism and that one of skill in the art would not be motivated to combine the teachings of Casey et al. with those of Fell et al. and Rajewsky et al., since there would be very little expectation for success given the potential folding problems that occur with such scFv molecules, let alone a larger construct comprising a full length heavy or light chain immunoglobulin molecule fused to a fluorescent protein such as EGFP. The task of making a genetically engineered non-human mammal whose genome has been altered such that the immune cells of said mammal secrete a detectably labeled fusion polypeptide (antibody) that is capable of proper folding and thus maintaining the specific binding characteristics, in addition to being detectably labeled, would seem daunting given the statements by Casey et al. This is particularly true given the larger size of the chimeric antibodies taught by Fell et al. and Rajewsky et al. These teachings, when combined, would lead one skilled in the art to believe that there would be very little chance of successfully generating a genetically engineered non-human mammal whose immune cells would be capable of secreting antibody molecules which retain their specific binding characteristics and functionality.

It was not until Applicants' present invention that an *in vivo* method for the production of genetically engineered non-human mammals that produce a chimeric antibody containing detectable proteins in response to a preselected antigen was possible. Furthermore, it was not until the present invention that genetically engineered non-human mammals or cells were produced that could respond to a single or multiple (different) antigenic challenge(s) and respond by secreting detectably labeled antibodies following such antigenic challenge(s). In addition, there have been no teachings prior to the present

invention, which demonstrated that mammals or cells could be engineered to produce antibodies labeled with either one detectable label or a plurality of labels. The advantages of such an animal or cell were not apparent until the time of the present invention. It was only through Applicants' work whereby it could be appreciated that a non-human mammal or cell derived from said mammal could be used repeatedly for multiple and different antigenic challenges/immunizations and each time respond by producing a specific antibody that has a single label or multiple labels, and thus could be used concomitantly for different purposes, for example, for ease of purification of the antibody or for detection of antigen in tissue specimens. Based on the difficulties apparent to those skilled in the art prior to the time of the present invention for producing such a genetic construct, no one could have envisioned that such construct could result in expression of an antibody that not only maintains its specific binding characteristics, but also retains its single or multiple labels for detection of antigen in tissue samples or for purification purposes etc., thus resulting in an antibody that provides multiple functions.

3. Declaration under 37 C.F.R 1.132 and References in Support of the Patentability of the Invention

Applicants submit a Declaration under 37 C.F.R 1.132 of Dr. Simon setting forth facts in support of the patentability of the claimed invention. As noted in the Declaration submitted herewith, the Declarant provides the names and cited references of several investigators who have tried to produce detectably labeled proteins, which are able to maintain function after labeling, and have failed. Several of these references have been submitted herewith with the declaration, and are labeled as EXHIBITS B through G.

For example, Han et al (J. Biological Chemistry, (2005), Vol. 280, No. 6, pp. 5089-5100, EXHIBIT B in the declaration) developed a strategy for labeling presynaptic terminals in transgenic mice. In particular, the investigators created transgenic mice expressing a presynaptic molecule, synaptotagmin 1, labeled with enhanced cyan fluorescent protein (ECFP) under the control of the Thy1 promoter. As the authors point out, a potential problem in labeling synapses with ECFP fusion proteins is that such proteins may have functional effects on synapses. To address this issue, they examined

the functionality of the synaptotagmin 1-ECFP fusion protein. To summarize, their data demonstrated that "transgenic mice expressing synaptotagmin 1-ECFP are useful tools for following presynaptic terminals *in vivo* but that the ECFP fusion inactivates synaptotagmin 1 functionality and thereby the synaptotagmin ECFP fusion protein is only a label at synapses without inserting itself as a functional component into the fusion machinery". *See* Simon Declaration, paragraph 7, EXHIBIT B. Accordingly, this reference supports the fact that attachment of a detectable label to a protein may sometimes lead to loss of function of the protein.

In addition, Pelkmans et al. (Nature Cell Biology (2001), Vol. 3, pp 473-483, in order to analyze caveolar endocytosis in living cells, investigated SV40 internalization using dual-color, video enhanced live microscopy with Texas Red-labeled virus and green fluorescent protein (GFP)-tagged caveolin-1 and tubulin. To visualize the distribution of caveolin-1 in live cells, they constructed plasmids encoding enhanced GFP at either the N or C terminus of canine caveolin-1. They determined that when cells were transfected, a similar distribution of fluorescent caveolin-1 was observed for both fusion proteins. However, they also noted that "whereas cells expressing C-terminally GFP-tagged caveolin-1 allowed normal SV40 infection, N-terminally tagged caveolin-1 served as a dominant negative inhibitor. It prevented uptake of SV40 into cells and inhibited expression of T-antigen." Thus, this experimental data serves as proof that GFP labeling of proteins does not always produce a normally functioning protein. That is, in certain situations, the tagging process itself can result in generation of a dominant-negative repressor molecule that inhibits the normal function of the protein. *See* Simon Declaration, paragraph 10, EXHIBIT C.

In addition, Wasle et al. (Biochem. J. (2004), 380: 897-905) examined the effect of expression of syncollin in AtT-20 neuroendocrine cells. Efficient expression was achieved by infection of the cells with adenoviral constructs encoding either untagged or GFP-tagged syncollin. The authors demonstrated that both forms of the protein were sorted into corticotrophin (ACTH)-positive secretory vesicles present mainly at the tips of the processes. Neither form affected basal corticotrophin secretion or the constitutive secretion of exogenously expressed secreted alkaline phosphatase. However, regulated

secretion of corticotrophin was inhibited by untagged but not by GFP-tagged syncollin. In parallel, untagged syncollin caused a 46% reduction in the number of secretory vesicles present at the tips of the cell processes. Syncollin-GFP was without effect. Thus, once again, it appears that the addition of a GFP tag to syncollin completely abolished its ability to perturb the later stages of the secretory pathway in AtT-20 cells. Since there appears to be no gross biochemical differences between the two forms of the protein that might account for the significant difference in their functional effects, the authors postulate that the presence of the GFP tag may have a significant effect on the structure of the syncollin homo-oligomer that might interfere with its ability to form pores in the structure. Thus, this is further proof that the attachment or tagging of a protein with GFP does not always provide for a functional protein. *See* Simon Declaration, paragraph 11, EXHIBIT E.

Further proof of lack of function of GFP fusion proteins can be found in Doyle and Botstein, (Proc. Natl. Acad. Sci. 93(9):3886-3891, 1996), whereby they demonstrated that actin fused to GFP does not function. These studies were done using time-lapsed movies which demonstrated the dynamic nature of cortical actin patch movement. Whereby normal patch movement is rapid in the cytoplasm, the movement of actin cortical patches with actin-GFP is constrained. *See* Simon Declaration, paragraph 11, EXHIBIT F.

Further proof of the possible negative effects of GFP labeling on protein function can be found in the abstract by German-Retana et al. (Mol. Plant Microbe Interact. (2000) Vol. 13(3):316-324, whereby the authors engineered the RNA genome of a resistance-breaking isolate of Lettuce mosaic virus (LMV-E) to express the jellyfish green fluorescent protein or beta-glucuronidase fused to the helper-component proteinase (HC-Pro) to study LMV invasion and spread in susceptible and resistant lettuce cultivars. The authors found that unlike the parental virus, the recombinant viruses were not able to overcome the protection afforded by the resistance mo1 gene. Thus, the authors conclude that "GUS or GFP tagging of the HC-Pro of LMV has significant negative effects on the biology of the virus, abolishing its resistance-breaking properties and reducing its pathogenicity in susceptible cultivars." *See* Simon Declaration, paragraph 13, EXHIBIT G.

Accordingly, it would appear that one of skill in the art who is familiar with the potential pitfalls associated with tagging a protein molecule with a detectable label such as GFP, while at the same time maintaining functionality, would not be motivated to attempt producing cells or transgenic mice such as those described in the present invention, since the chance of achieving success was questionable. Thus, with such a lack of motivation to succeed, Applicants assert that the skilled artisan would not have combined the teachings of Fell et al. in view of Casey et al to produce cells containing and secreting a detectably labeled antibody such as that disclosed in the present application. Furthermore, taking into account the numerous references (only a few of which are described above) that teach a loss of function upon tagging a protein molecule with a detectable label such as GFP and combining this knowledge with that of basic immunology which describes the numerous rearrangement reactions that occur during the generation of antibodies by B cells and the immune system, it is unlikely that a skilled artisan would be motivated to attempt the preparation of the cell and animal constructs of the present invention. Thus, even when taking into account the teachings of Fell et al. in view of Casey et al. and further in view of Rajewsky et al. it is unlikely that a skilled artisan would be motivated to attempt such a task given the low probability for success.

Based on the foregoing arguments and claim amendments, withdrawal of the rejection is respectfully requested.

***Fees***

A check in the amount of $905.00 is enclosed to cover the Request for Continued Examination and a petition for a three month extension of time. No other fees are believed to be necessitated by the foregoing response. However, if this is in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or credit any overages.

***Conclusion***

Applicants believe that the foregoing amendments to the claims and arguments place the application in condition for allowance. Withdrawal of the rejections is respectfully requested. If a discussion with the undersigned will be of assistance in resolving any remaining issues, the Examiner is invited to telephone the undersigned at (201) 487-5800, ext. 118, to effect a resolution.

Respectfully submitted,



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Attachments: Request for Continued Examination; Petition for a three month extension of time; Declaration under 37 CFR 1.132 and Exhibits A-G.